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Novel Bacterial Isolate For Amylase Production, Characterization And Application On Textile Using Saffron Natural Dye

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Abstract

The screening experiments from soil samples led to the isolation of some amylase-producing bacterial isolates. The bacterial isolate showed maximum amylase production was selected for further physiological and biochemical investigations. The results showed that the maximum activity (125u/ml)was obtained by using a fermentation medium consisting of (g/l) starch,10 peptone 10 yeast extract 5, ammonium sulphate 5, magnesium sulphate 0.25, CaCl2 0.25 between 80 1ml at pH 5.6,fermentation time 48 h at 300C.The obtained amylase underwent partial purification using ammonium sulphate at conc.40wt%.The purified enzyme was applied to wool fibres dyed with saffron natural dye. The phytogenic analysis of the isolated strain appeared to agree with the Bacillus mycoides. The results obtained exhibited higher colour strength for the treated samples compared to the untreated fibbers.

Key words: Amylase, producion, characterization, application, textile

Introduction

Amylases (Ec.3.2.1) are a group of hydrolysis enzymes that catalysis the hydrolysis of amylase and amylopectin from starch and other derivatives [1]. They showed great significance in many industrial applications. This class of enzymes play an important role in recent biotechnology [2].It constitutes about 33% of the world industrial enzymes market. They possess a wide performance application in different industrial processes especially saccharification in textile, food, baking, brewing, and distilling industry [3]. Amylases have been produced by many sources animals, plants, microbes specialty yeast, bacteria fungi as well as some actinomycetes [4].

Application of natural dyes showed that they are eco-friendly. They appeared to be more gentle in the skin as well as applicable for most types of fabric industries[5.]. Natural dyes could be obtained from plants, vegetables, and many other sources. The most important and valuable source of natural pigments are the microbial sources due to their high production, easy cultivation n a large scale more economical than other sources.

They are also revealed antimicrobial activity, not allergic to the skin and eyes [6].Several research publications have also concluded the application and antibacterial properties of natural dyes [7].The aim of the present studies was to select a potent new isolate for amylase production, investigate some physiological and biochemical factors affecting the production process, enzyme partial purification as well and application in the textile industry. The environmental precautions and safety demonstrate the suitability of using natural dyes since it found to be eco-friendly materials ion using in different applications. It was found to be nontoxic, save compared to synthetic pigments

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O **Materials and methods**

Microorganism

The bacterial isolate used in the current work was selected from different soil samples obtained from a farm in Giza governorate under sterile conditions and transferred under sterile conditions to the laboratory. After serial dilution samples were used inoculated to previously sterilized nutrient agar medium and incubated at $30C^0$for 24h. The colonies obtained were

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investigated and the pure one was re-cultured on a nutrient agar slant at stored at $4C^0$[8]. The selected isolate was undergo biochemical and phylogenic identification it was found to be matched with *Bacillus mycoides.*

2.2-Inoculums preparation

The pure colony of the isolated bacteria was kept on agar slants at $4C^0$ and used to inoculate an Erlenmeyer flask 250ml containing 100ml nutrient broth and incubated for 24 h. at $30C^0$ at 200rpm.

2.3-Fermentation process (α-amylase production)

The enzyme production process was carried out using the fermentation medium consistes of(g/L) starch 10, peptone 10, Yeast extract 20, $MnCl_2$ 0.015, $CaCl_2$ 0.05 KH_2PO_4 0.05, $MgSO_4$ 0.25, and $FeSO_4$ 0.01 the pH was maintained at 7. It was sterilized by autoclaving at the recommended conditions. The production media was inoculated under aseptic conditions by 2 ml inoculum of the bacterial strain, incubated at 35°C for 48 h. After incubation the fermented broth was subjected to centrifugation at 10000 rpm for 10min at 4°C[8]. The clear supernatant was used as an enzyme source for further activity measurement.

2.4-amylase assay

The crude filtrate obtained after centrifugation was assayed for activity by measuring the release of reducing sugar according to the modified method ofFisher and Stein, [9] where it was achieved by using DNS methods and OD measurement carried out at 540 nm.

2.5-Molecular identification

The genetic identification of the bacterial isolate involved 16S rRNA gene sequencing. PCR amplification was done using Maxima Hot Start PCR Master Mix (Thermo K1051) in Sigma Company of Scientific Services. The analysis of phylogeny results and comparison with sequencing deposit it was found to be *Bacillus mycoids*.

2.6-The process of Dyeing

Various concentrations (20-80 g/l) of saffrondye usinga liquor ratio 1:100.Wool fibres without treatment and after treatment with enzyme is subjected to dyeing using the microwave at pH 5 at various time intervals(1-5 min)[10].The fibres were washedwithwarm water and then cold water.Washing carried out in a bath containing 5g/l non-ionic detergent at 50ºC for 30 minutes, then washed and dried in air at ambient temperature.

2.7-Measurements of colour strength (K/S value)

An Ultra Scan PRO spectrophotometer was used to estimate the reflectance. K/S value was tested spectrophotometrically at wavelength 500 nm. The K/S values of untreated and pre-treated wool fibers with enzyme were then determined.

3-Results and Discussion

3.1-Fermentation time

The effect of different times (24,48,72,96 and 120 h) on the production of alpha-amylase were investigated and the results (fig.1)indicated that the best time was at 48h. after which decreasedand reached to minimum value at 120h[10,11].

It was found that rise of the fermentation period [12] causes a decline in the production of amylase enzyme which may be due to the formation of other by-products and the diminishing of the carbon and nitrogen sources. These substances may inhibit bacterial growth.

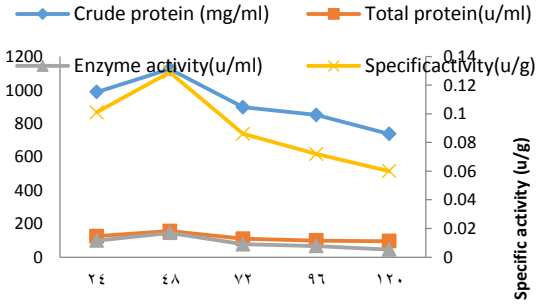


Figure .1 Effect of different fermentation time on the production of amylase enzyme

3.2-Temperature/ amylase activity relation ship

The effect of different temperatures (25,35,45,55 and $65C^0$) on the activity were tested and the results (fig.2) showed that the maximum activity was at $45C^0$.The activity was reduced at the temperature degrees above this value and the minimum activity was obtained at 65 C^0[13].Cultivation temperature is very important for bacterial growth which varies according to the bacterial sp. It was found that temp affects on enzyme synthesis process and energy transfer system of bacterial cells [14].The optimum growth temperature of *Bacillus subtilis* KC3 was around 33°C^Onthe other hand, the crude amylase of it was found optimally active at 50°C.

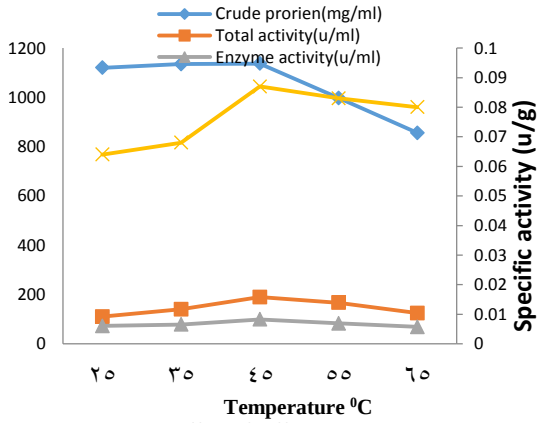


Figure. 2 Effect of different temperature on the production of amylase enzyme

3.3-Effect of different pH values on the production of amylase enzyme

The effect of different pH value of the fermentation medium (5,6,7,8 and 9) on the production of amylase was investigated. The Results in (fig. 3) showed that the bestenzyme production (0.239u/ml) was obtained at 8. However, the yield was reduced (0.154u/ml) at pH 9. These results correlated with those obtained by [15]. The enzyme productivity was greatly affected by pH value of the surrounding medium.pH value of the fermentation medium affects the enzyme activity, the morphology of the cytoplasmic membrane , products construction as well as oxidation reduction reactions[16].

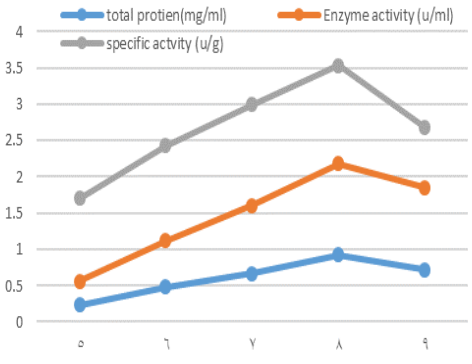
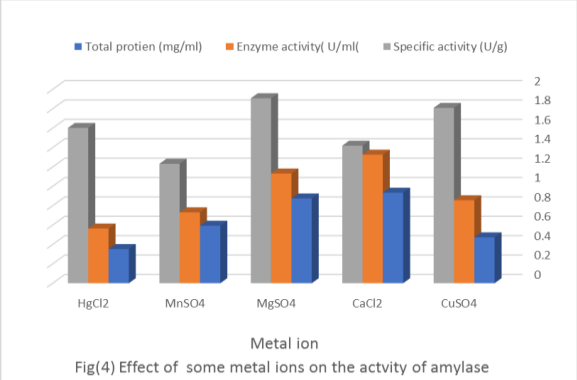


Figure .3 Effect of different pH value of the fermentation medium on the production of amylase enzyme

3.3-Effect of some metal ions

In the present experiment, the effect of different metal ions ($CuSO_4$, $CaCl_2$, $MgSO_4$, $MnSO_4$, and $HgCl_2$) was tested. The data in (fig. 4) revealed that the best amylase activity was obtained by using $CaCl_2$ as well as $MgSO_4$(0.126 and 0.113 u/ml respectively).Meanwhile, moderate activity was recorded by using $CuSO_4$(0.811u/ml).On the other hand, by using $MnSO_4$ and $HgCl_2$ reduced activity were noticed (0.612 and 0.521u/ml) respectively. [17]



Fig(4) Effect of some metal ions on the actvity of amylase

3.4-Purification using ammonium sulphate

The broth was centrifuged at 10,000 rpm for 15 min at a temperature 4°C. The enzyme protein ofthe supernatant was concentrated.[18,19]. The supernatant with enzyme was chilled, and solid ammonium sulfate was added with gentle stirring to 20% saturation; after centrifugation, the precipitate was discarded. The process was repeated to reach the other saturation (40, 60,and 80%). The precipitate was collected and dissolved in 0.01 M sodium phosphate buffer (pH 6.4) and dialyzed overnight against the same buffer. The best purification was obtained at 60% Estimation of protein was estimated according to the method of Lowry [20]with crystalline bovine serum albumin (Sigma Chemical Co.) as the standard.

Fractionation of crude enzyme was carried out using five saturations of ammonium sulphate percentages (20,40,60 and 80%). The results showed that the best enzyme activity(67u/mg) was obtained at a saturation 40%. On the other hand, the other saturation showed reduced activity.

3.5-Phylogenic tree of the isolated bacteria

The result obtained by the phylogenic analysis showed that it agrees with bacillus mycoides strain.

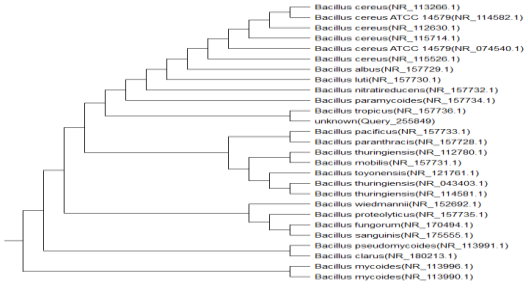


Figure .5. Phylogeny tree of the isolated bacteria

3.6-Effect of amylase concentrations on wool fibers dyed with saffron natural dye

The results of this study indicated that, the pre-treatment using (2–.10 %) conc. of amylase exhibited highest values of colour strength (K/S) at 8% concentration of wool fibre dyed with saffron natural dye by microwave as appeared in (fig.6)and table (2).

Table 2. Effect of conc. of amylase on K/S and color difference values for wool fibers dyed with saffron natural dye dye by microwave method.

Conc. g/L.	K/S	L*	a*	b*	C*	h	ΔE
0%	20.00	39.44	65.93	27.89	63.39	26.10	74.66
2%	25.35	39.43	57.48	28.83	64.30	26.64	75.43
4%	31.83	37.77	56.46	28.02	63.03	26.39	73.48
6%	29.54	38.15	65.80	27.94	63.30	26.19	73.91
8%	44.52	37.18	50.52	20.19	54.41	21.78	65.90

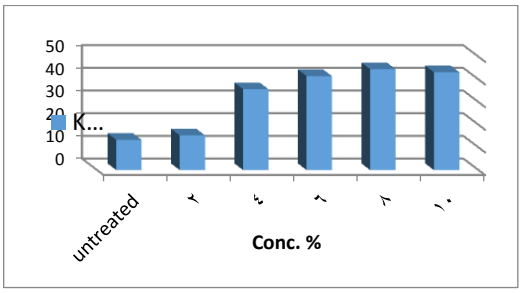


Figure .6 Effect . of enzyme concentration on the colour strength (K/S) for wool fibres dyed with saffron natural dye

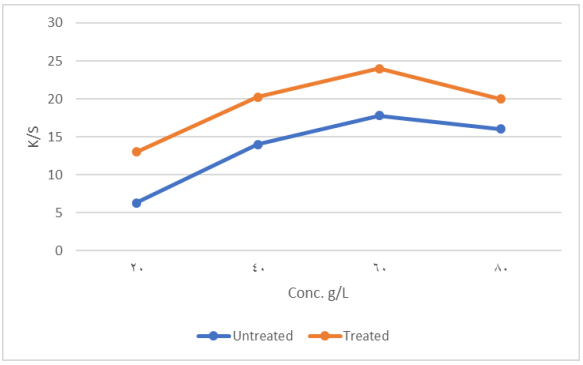


Figure .7 Effect of dye concentration on the color strength(K/S)

3.7-Effect of dye concentration

Saffron dye was used for dyeing wool fibers at various concentrations (20- 80 g/l) of a liquor ratio1:100 [20,21] .Dyeing was carried out using microwave within 5 min.The results showed that the color strength values were increased when the concentration of the dye increasedto60g/l on drying by microwave method (fig7).

Microwave heating instrument depending on ionic conduction. It is a kind of resistance. Depending onthe accelerating of the molecules in the dyeing bath, This leads to the crashing of dye molecules by the particle of the fibre [21]. The dielectric character of microwave refers to the intrinsic electrical characteristics that affect dyeing by the dipolar spin of the colour and effectiveness of the microwave field on the dipoles. Various concentrations of the dye were done by microwave.

The results showed that by increasing dye concentration L* value is decreased and the color of the dyed fibres becomes darker. By increasing the dye concentration, a* and b* amounts increase in the positive line. The wool samples colour changed into reddish yellow colour and got darker with increasing the dye concentrations in the range of (2-8) g/ L.

3.8-Antimicrobial activity

Different enzyme concentrations(2,4,6,8,and 10%) were tested for their antimicrobial activity towered gram negative bacteria E coli, gram-positive bacteria (*B, subtilus* pathogenic yeast (*C. albican* and *Staphylococcus aureus*) [22,23]. The results presented in Table (2) showed that it was priority effective against gram-negative and gram positive bacteria. But show a slight effect on pathogenic bacteria as well as moderate effects on *Staphylococcus aureus*.

Conclusion

The isolation of a new bacterial strain for amylase production accompanied by biochemical and phylogeny identification revealed that the isolate agree genetically with the *Bacillus mycoides* strain. Investigation of the physiological and fermentation parameters affecting the amylase production, the maximum enzyme production was obtained by using a fermentation medium consisting of starch,10 peptone 10 yeast extract 5, ammonium sulphate 5, $MgSO_4$.7 H_2O 0.25, $CaCl_2$ 0.25 between 80 1ml at pH 8,fermentation time 48 h at $45C^{0.}$

Table .3 Antimicrobial activity of the obtained enzyme

Enzyme con. %	Inhibition Zone(cm)			
	E .coli	***B.subtilus***	***C. albicans***	***S.aureus***
Control	0.5	0.4	-	-
2 %	1.5	1.1	1.2	1.4
4 %	2	1.3	1.2	1.5
6 %	2.7	1.4	1.3	1.6
8 %	1.7	0.9	1.1	1.2
10 %	1.7	0.9	1.1	1.2

Control wool sample without enzyme treatment

The results also indicated the priority of using the salts $CaCl_2$ as well as $MgSO_4$ for enzyme production.The results also show that the K/S was increased by increasing the concentration of the dye to60g/l on dyeing by microwave method. On the other hand, the antimicrobial activity showed that it was more effective against gram-negative than gram positive bacteria as well as pathogenic yeast.

Conflict of interest

There are no conflicts of interest**.**

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